March 6, 1949.

Dear Max,

I have the cultures you sent, and your very impressive pedigree of 2/26 A. Your numbering scheme is very ingenious, and will make it much more painless to discuss what comes up. To be sure that I've gotten it straight, I'm sending back the exapnded line pedigree, together with certain inferences.

First the pedigree. The critical cell divisions seem, of course, to be those of 3 and 5. 5 seems to have segregated at its division to give what may be inferred to be a haploid segregant (11) which thereafter breeds true (47-48; 99-102), and a diploid heterozygote which divided only equationally (12 -- 25,26 -- 51; 219-222) for the next 4 generations. I've looked at the 6 segregants a little further and was a little surprised to find that they were all Xyl+ V_1^r. I probably will have more to say about A51, but it seems to be unusual in being Xyl-variegated (-v), but with + predominant to -. My past experience with H-72 has been that - predominated over + about 13:1 among the segregants, and I have also never seen a change of type before in the diploids. This may be an exceptional example of crossing-over without reduction, but I'll have to look at it some more. If this happened before 5, it may explain why all the 6 segregants are the crossover type Lac-Xyl+. Otherwise, this type of behavior might call for two-strand crossing over (or else that we had a rare four-strand double). I would have expected, on a 4-strand basis, that a cell like 11, if crossing-over occurred, might give one crossover, 23, Lac-Xyl+, and one non-crossover, 24, Lac-Xyl-. But it is unsafe to try to generalize on just one observation, and 5 may have changed type from H-72.

How a cell, 5, can segregate to one haploid and one diploid product may not be easy to explain, but perhaps we can appeal to the probable multinucleate condition of coli cells. (Cytological work on H-72 has been started. If the "Robinow bodies" are nuclei, this presumption is justified). Then, we can imagine that in a binucleate cell, one nucleus might segregate, the other remain diploid. Of the segregants, one pair carries a lethal, which shows up in the 6 descent; the other does not segregate from the diploid until 5 divides. From this point of view, the ternary fissions that you mentioned would be especially interesting. But this kind of explanation can be made to suit almost any segregation pattern. I hope that some simpler patterns will turn up.

I think your procedure of recovering microcolonies at a very early stage is very well advised, and removes one of the more important anxieties I had about this program.

As to classification: Certainly, any cell which produces any mosaics must be a heterozygote. Likewise, any cell which is homogeneous over 100 - 1000 colonies is very unlikely to be heterozygous, although conceivably it could have been a heterozygote which segregated uniformly by the time you recovered it.

The cultures which have no mosaics, but mixtures of + and -, pose another problem, especially if there are nearly equal in numbers. Such cultures could represent heterozygotes which have segregated completely. They also might represent the first reduction division of a heterozygote, as could be verified by finding only two combinations of factors (including V^r, Xyl and nutrition) in the whole population.

The "lethal" cells are most interesting, as I have had to postulate them to account for the deviation of the Lac segregation ratio from 1 + : 1 -, to 1 : 7.5 for H-72. Enclosed is a draft of a manuscript that has been sent to PNAS going over this point.

When maintained on EMS, most Lac+ prototrophs are heterozygous. Only a few %, at most, will be prototrophic segregants.

I still haven't checked directly on the nutrition of H-72 segregants, but would infer that at least M, T, and L are heterozygous. I would also put biotin and thiamin into the testing media. These are difficult to score for, but chances should not be taken against the cultures being B- or B_1- . In testing the nutrition, simply add a drop of a dilute suspension of freshly grown cells (most conveniently scraped from nutrient agar) to 10 cc of minimal liquid medium supplemented as follows:

a. BB_1 MTL (+)
b. BB_1 MT (-L)
c. " ML (-T)
d. " TL (-M)

Lack of growth after 24-36 h. in -X denotes a requirement for X, provided there is adequate growth in the + culture. You will, of course, have to use well-cleaned glassware, and dilute inocula. Controls with known parents are desirable.

Reverse-mutation certainly does occur, and is pushed by selective pressure on lactose-EMB. Lederberg is studying this system. (Abstract reprint enclosed). It should cause no trouble except in cultures repeatedly transferred on EMB-Lac. Reversion has nothing to do with the segregation phenomenon.

I can see no pressing advantage to sending the original cultures, provided care is taken to include a complete sample (i.e., no fresh single-colony isolation). For now, I would appreciate getting the mosaic single-cell-isolates as well as the segregants, but this should not be necessary later.

You have the best estimate of the frequency of segregation in your pedigree. Out of 8 successful fissions of a heterozygote, there was one segregation. This seems quite reasonable from the sectored appearance of the colonies on EMB. Assuming that diploids and haploids grow at the same rate, this means, on a rough average, that the proportion of heterozygotes will diminish by 1/8 each fission cycle, i.e., that a heterozygote is lost every third generation. This means that the diploids will increase 7-fold each three mgt's, the haploids will increase 8-fold, plus one for each diploid. If h is the number of heterozygotes initially, and s the segregants,

$\frac{dh}{dt} = 7h$ $\frac{ds}{dt} = 8s + h$, where the time unit is three generations. I ran across these differential equations once before in connection with some yeast work, and one gets:

$$\frac{dh}{dn} = \frac{7}{8}\frac{h}{n}$$ where $n = h + s$ = total population.

and, for the boundary condition of a population starting from a single h cell, $h/n = 1/n^{8}$. That is, the proportion of h will be 10% when $n = 10^{8}$, which seems to be substantially correct. Having just gone through the algebra, it is apparent that the frequency of segregation is the reciprocal of the log population size for which the proportion of heterozygotes is diminished by the factor 1/10. This analysis excludes the lethals, but more information about them will be needed before a mathematical model can be set up.

H-72 is a prototroph from the cross mentioned in my letter Nov. 15. Succinate is put in EMS-Lac so that Lac- / prototrophs be detected as white colonies. You probably don't need to use it for your purposes.

I'm sending E. coli K-12, "58" which requires biotin. Bernard D. Davis, in New York, also has some such.

Sincerely,

Joshua Lederberg

P.S. Maybe it's about time we got together. Any suggestions?

Enc: MS
reprint.